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Research

New Distribution Record for *Lucilia cuprina* (Diptera: Calliphoridae) in Indiana, United States

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Abstract

Determining range expansion for insect species is vital in order to evaluate their impact on new ecosystems and communities. This is particularly important for species which could be potentially harmful to humans or domestic animals. *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) can act as a facultative ectoparasite and has an extensive history as the primary inducer of sheep-strike in Australia, New Zealand, and Africa. We present here the first record of this species in Indiana, United States. *Lucilia cuprina*'s range expansion northward in the United States may be indicative of changing environmental conditions conducive to the proliferation of this species into historically cooler climates. The presence of this species could significantly impact forensic death investigations utilizing dipteran larvae to estimate a minimum postmortem interval. If range expansion of this species is not taken into account by a forensic entomologist (especillay if *L. cuprina* is not known previously in their region), and if this species is misidentified as the closely related species *Lucilia sericata* Meigen (Diptera: Calliphoridae), an inaccurate minimum postmortem interval (PMI_{MIN}) estimation may be made. Therefore, the range expansion of this fly could have large impacts for many different entomological disciplines.

Key words: blow fly, myiasis, forensic

The genus *Lucilia* Robineau-Desvoidy (=*Phaenicia* Meigen, Diptera: Calliphoridae) constitutes a small group of blow flies often referred to as the greenbottles due to their shiny green appearance (Aubertin 1933). It is often difficult to differentiate between adult as well as larval congeners based on morphology as they share close resemblances to one another (Aubertin 1933, Whitworth 2006, Williams et al. 2016); thus, many researchers have turned to molecular methods as a means of identification (Stevens and Wall 1996a; Wells et al. 2007; DeBry et al. 2010, 2013; Nakano and Honda 2015; Yusseff-Vanegas and Agnarsson 2017). Though most species in this genus are carrion-breeders, some are facultative ectoparasites (i.e., myiasis producers), with the most notable being *Lucilia cuprina* Wiedemann (=*Phaenicia cuprina* Wiedemann, *Phaenicia pallescens* Shannon) and *Lucilia sericata* Meigen (Diptera: Calliphoridae). *Lucilia cuprina* is well-established as the primary myiasis producer of sheep in Australia (Tellam and Bowles 1997), New Zealand (Heath and Bishop 2006), and Africa (James 1947), whereas *L. sericata* is the main culprit of strikes in the United Kingdom (French et al. 1995) and other European countries. North American *L. cuprina* and *L. sericata* have rarely been implicated in myiasis in this region, though there has been one recorded case of human cutaneous myiasis by *L. sericata* in the United States (Sherman 2000).

As *Lucilia* spp. larvae are also carrion-feeders, this genus has utility in forensic death investigations in which they have colonized a corpse (Benecke 1998, Sukontason et al. 2007, Pohjoismäki et al. 2010). A handful of studies have investigated the developmental requirements for North American strains of *L. sericata* (Tarone and Foran 2006, 2008; Gallagher et al. 2010; Tarone et al. 2011), but only one similar study exists for North American *L. cuprina*, though this was under the synonym *P. pallescens* (Ash and Greenberg 1975b). Development data for *L. cuprina* primarily exist for other regions, including Australia, South Africa, India, and Sri Lanka (Day and Wallman 2006, Kotzé et al. 2015, Bansode et al. 2016, Bambaradeniya et al. 2017).

Lucilia cuprina is known to be economically, agriculturally, and forensically important, so it is prudent to keep track of its range. Its current distribution records range from Virginia to California and along several southern states (Whitworth 2006), though there are reports from the 1950s (but not since) observing this fly as far north as Michigan. We now present a new record for *L. cuprina* Wiedemann in Indiana, United States.

Methods

Adult fly collections were made from urban sites (mostly public parks) throughout Indiana from June – August 2015 and March – October in both 2016 and 2017 as part of a larger survey of blow flies (Diptera: Calliphoridae; unpublished) (Table 1). Decayed chicken liver was exposed for 20 – 30 min at each location, and flies were collected with an aerial sweep net and killed in 70% ethanol on-site. Temperature data were collected at each site using a Datalogging RH/Temperature Pen (SPER Scientific, Scottsdale, AZ) elevated ~1 m above the ground and archived precipitation data from the collection date, the day before collections, and the week prior to collections was obtained. Historical mean temperature data spanning from 1940 to 2017 were also obtained for Indiana and the Midwest as a whole from the online data portal, cli-MATE (Midwestern Regional Climate Center 2018), as this range encompasses the time period in which *L. cuprina* were collected in the Midwest.

Lucilia cuprina were identified using a dichotomous morphological key (Whitworth 2006). Select specimens are vouchered at the Purdue University Insect Collection, West Lafayette, IN. Additionally, data from previous developmental studies of *L. sericata* and *L. cuprina* were gathered for comparison purposes. Average minimum total development times (i.e., egg to adult eclosion) from these studies were obtained and transformed into accumulated degree hours (ADH) for comparison purposes. In order to show the potential for error when *L. sericata* development data are used instead of *L. cuprina* data (as in the case of a forensic entomologist misidentifying larval specimens from a forensic investigation), a percent error was generated for when *L. sericata* data are used when the specimens in question are actually *L. cuprina*. This calculation was performed by taking the absolute value of the difference between ADH of the reference population (*L. sericata*) and the actual population (*L. cuprina*), dividing this value by the ADH for the actual population, and then multiplying by 100. Even though flies from each study were obtained from many different regions and populations, and reared under different conditions, they represent the data sets that would be used by a forensic entomologist during a death investigation.

Results

In total, 28 individuals of *L. cuprina* were collected from eight different localities in central Indiana from 2015 to 2017 (Table 1; Fig. 1). Province Park and Northwest Park were only added as collection sites in 2016, and thus were not sampled in 2015. Out of the 15 time points in which *L. cuprina* was collected, only 6 dates yielded more than one specimen per collection site. Only one instance (12 July 2017, Province Park) was recorded in which both male and female specimens were collected together, as all other collections only consisted of single sex samples. Though a majority ($N = 23$) of flies were collected in the warmer summer months (mean = 30.2°C), this fly was collected at temperatures as low as 12.7°C. Of the 28 total specimens collected, only five were collected in the cooler spring and fall months (mean = 21.1°C). Archived precipitation data revealed negligible precipitation (i.e., 0.00 cm at nearly all data points) leading up to all *L. cuprina* collections.

Mean temperature data comparisons of Indiana and the entire Midwest between the years of 1940 and 2017 are also given (Fig. 2). Indiana maintained an average 1.88 ± 0.25°C warmer mean temperature than the whole Midwest region during this time period (1940–2017). Though there are regular oscillations in temperature for both regions over the 77-yr span, both regions show a slight increase in temperature (~1°C) over this time period. In 1953, 2 yr before the Schoof and Savage (1955) study in which *L. cuprina* was collected abundantly from Muskegon, MI, mean temperatures for the Midwest and Indiana peaked (9.89 and 11.89°C, respectively) and then experienced a downward trend until 1959. The temperature

Table 1. Collection dates and geographic locations for *L. cuprina* collected in Indiana, United States

Site	City	Latitude, longitude	Date	Avg temp (°C)
Military Park[a]	Indianapolis	39°46′16″, −86°10′08″	2 July 2015	26.5
			14 August 2015	29.9
			3 August 2016	29.9
			31 August 2017	24.1
			13 October 2017	21.1
Skiles Test Park[a]	Indianapolis	39°52′21″, −86°29′50″	25 May 2016	28.5
Broad Ripple Park[a]	Indianapolis	39°52′17″, −86°07′51″	25 May 2016	28.5
			19 April 2017	27.9
			12 July 2017	31.0
University Park[a]	Greenwood	39°36′36″, −86°03′02″	4 July 2015	24.2
			23 June 2016	30.8
			13 October 2017	22.7
Northwest Park[b]	Greenwood	39°37′42″, −86°08′36″	14 June 2017	33.1
Province Park[b]	Franklin	39°28′37″, −86°06′39″	25 May 2016	28.5
			23 June 2016	31.2
			7 July 2016	26.8
			13 October 2016	12.7
			12 July 2017	33.2
			26 July 2017	32.7
Near Restaurant[c]	Seymour	38°57′33″, −85°50′52″	30 August 2015	33.6
Near Otis Park Golf Course[c]	Bedford	38°51′32″, −86°27′38″	4 August 2015	37.5

Geographic coordinates are given as degrees, minutes, seconds (dms). Temperature (°C) data were collected on-site.
[a]Site used in 2015, 2016, and 2017.
[b]Site used in 2016 and 2017.
[c]Site used in 2015 only.

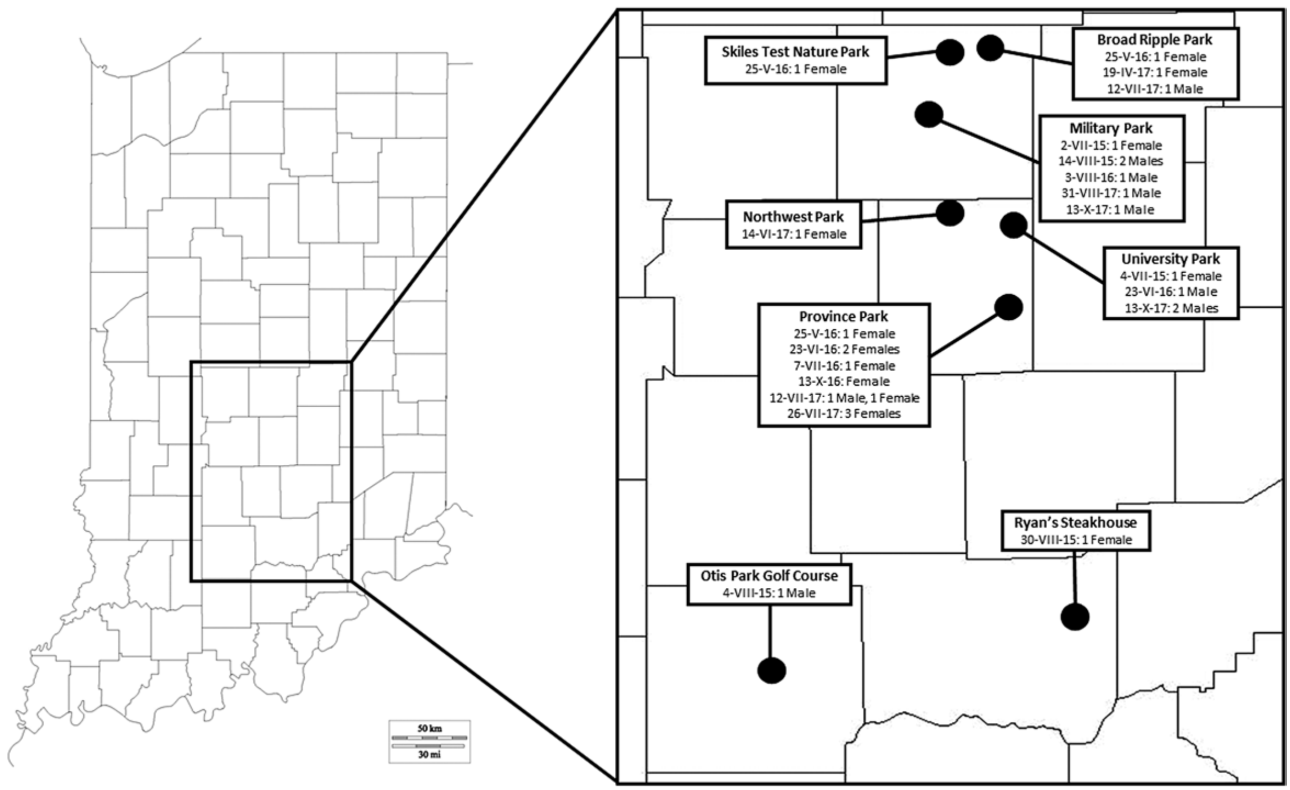

Fig. 1. Annotated map of Indiana, United States with collection information given. For each site, the date of collections, number of flies, and sex of individuals are displayed. Base layer of map obtained online (d-maps.com 2018).

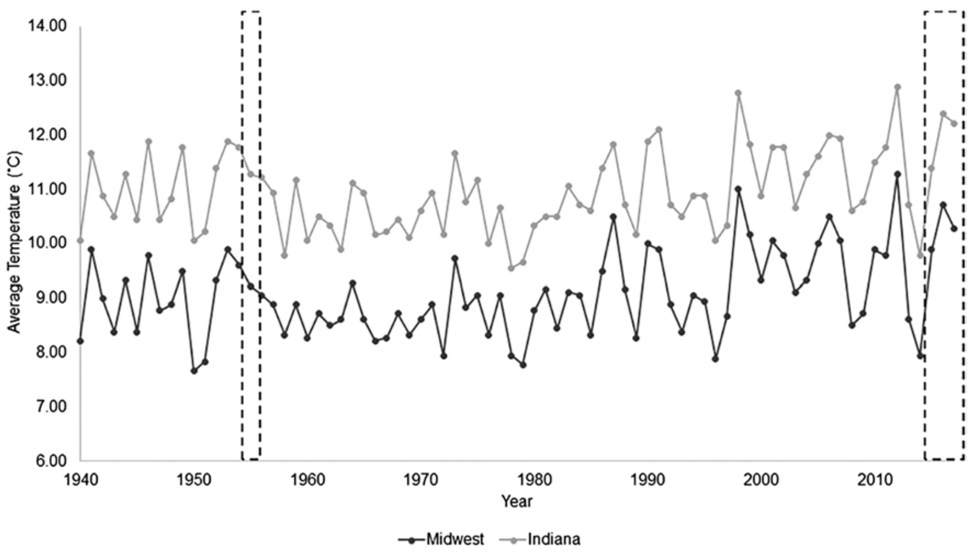

Fig. 2. Mean temperature (°C) comparison for the entire Midwest (black) and Indiana (gray) from 1940 to 2017. Dashed-line boxes enclose data for *L. cuprina* collected from Michigan in 1955 (left box, Schoof and Savage 1955), and from the current study in Indiana from 2015 to 2017 (right box).

trend leading up to 2015 (the first year *L. cuprina* was detected in Indiana, Table 1) shows a sharp decrease from 2012 to 2014 (11.28 to 7.94°C in the Midwest, 12.89 to 9.78°C in Indiana), with 2015–2017 experiencing increasing temperatures (9.89 to 10.28°C in Midwest, 11.39 to 12.22°C in Indiana).

Published developmental data for *L. cuprina* show that this species exhibits a longer average minimum total development time (663 h, 9,034 ADH) when compared with similar published data sets for *L. sericata* (467 h, 6,607 ADH) (Table 2). All *L. sericata* strains investigated exhibited similar minimum development times regardless of whether they originated from the United States (Tarone et al. 2011), Canada (Anderson 2000), or Austria (Grassberger and Reiter 2001) (Table 2). Both Sri Lankan (Bambaradeniya et al. 2017) and Indian (Bansode et al. 2016) *L. cuprina* strains were similar in development time, though the U.S. strain (Ash and Greenberg 1975b) exhibited the longest development time of all strains. When *L. sericata* data are used to estimate a PMI_{MIN} with *L. cuprina* specimens, percent error can range between 17.90% and

Table 2. Comparison of developmental data sets for *L. sericata* and *L. cuprina*

	Study	Region of flies	Temperature (°C)	Development time h (ADH)
L. sericata	Tarone et al. (2011)	California, United States	20.0	458.9 (6,424.6)
	Tarone et al. (2011)	Michigan, United States	20.0	463.9 (6,494.6)
	Tarone et al. (2011)	West Virginia, United States	20.0	475.5 (6,657.0)
	Anderson (2000)	Canada	20.7	486.2 (7,147.1)
	Grassberger and Reiter (2001)	Austria	20.0	451.0 (6,314.0)
L. cuprina	Ash and Greenberg (1975b)	Florida, United States	19.0	739.9 (9,619.0)
	Bambaradeniya et al. (2017)	Sri Lanka	20.0	621.8 (8,705.7)
	Bansode et al. (2016)	India	20.0	627.0 (8,778.0)

Region indicates where the flies used in each study originated, temperature refers to the temperature (°C) at which the developmental study took place, and the minimum development time indicates the time interval initiating at the egg stage and ending at adult eclosion. Minimum development time is given in hours with accumulated degree hours (ADH, 6°C minimum threshold temperature) in parentheses.

Table 3. Summary of mean percent error (%) generated when reference data sets of both *L. sericata* and *L. cuprina* are used to determine the minimum development rate of *L. cuprina*

		Hypothetical *L. cuprina* populations			
Species	Reference data set	Similar to United States	Similar to Sri Lanka	Similar to India	Mean % error (SD)
L. sericata	Tarone et al. (2011)—CA	33.21%	26.20%	26.81%	26.71 (4.76)
	Tarone et al. (2011)—MI	32.48%	25.40%	26.01%	
	Tarone et al. (2011)—WV	30.79%	23.53%	24.16%	
	Anderson (2000)	25.70%	17.90%	18.58%	
	Grassberger and Reiter (2001)	34.36%	27.47%	28.07%	
L. cuprina	Ash and Greenberg (1975b)	0.00%	10.49%	9.58%	6.66 (4.55)
	Bambaradeniya et al. (2017)	9.50%	0.00%	0.82%	
	Bansode et al. (2016)	8.74%	0.83%	0.00%	

Percent error values were generated using the following formula: (|ADH hypothetical *L. cuprina* population − ADH reference data|/ADH hypothetical *L. cuprina* population) * 100. CA = California, MI = Michigan, and WV = West Virginia *L. sericata* populations investigated by Tarone et al. (2011).

34.36% (mean = 26.71% ± 4.76%). Alternatively, using the wrong conspecific regional data for *L. cuprina* can result in 0.82–10.49% (mean = 6.66% ± 4.55%) error (Table 3).

Discussion

There have been no previous records of *L. cuprina* in Indiana, United States until now, thus representing a new species distribution record for this state. Furthermore, with this new record, it is likely other locations with similar temperatures may be experiencing the same range expansion. Though *L. cuprina* spans multiple continents, including Europe, Australia, Africa, and some parts of North America (Stevens and Wall 1996b, 1997), its distribution in the United States tends to be limited to warm regions, including California (Brundage et al. 2011), Florida (DeBry et al. 2010), Georgia (Googe 2014), Virginia (Hall and Townsend 1977), West Virginia (Mail and Schoof 1954), Kansas (Schoof et al. 1956), Arizona (Siverly and Schoof 1955, Sherman 2000), Texas (DeBry et al. 2013), and Missouri (Whitworth 2006), though there is one previous record of this species being collected as far north as Michigan (Schoof and Savage 1955). It should also be noted that extensive fly surveys conducted in Morgantown, WV in the 2000s (personal communications) yielded no *L. cuprina* specimens. Furthermore, extensive arthropod collections from pig carcasses (N = 53) in Rensselaer, IN (approximately 165 km northwest of Indianapolis, IN) from 2008 to 2010 did not record any *L. cuprina* in this region (Perez et al. 2014). Since the collection periods for our study and the Perez et al. (2014) study do not overlap, it is possible that 1) *L. cuprina* may have arrived in Indiana after 2010, 2) their range did not extend as far north in Indiana as Rensselaer at the time of that study, or 3) *L. cuprina* populations were not large enough or able to compete with local species at the pig carcasses; thus, they were not detected by the investigator. Multiple additional fly collections were made in Rensselaer using decayed liver bait in June–September 2015, yet no *L. cuprina* specimens were collected (unpublished data). Therefore, it is most likely that this fly has not yet extended its range to the most northern part of the state.

With recent increases in temperature and rainfall over the last century (NOAA National Centers for Environmental Information 2017, Midwestern Regional Climate Center 2018), it is not surprising that *L. cuprina* may have expanded its range northward. Recently, the first occurrence of the invasive oriental latrine fly, *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae), was recorded in Indiana, United States (Picard 2013), with subsequent collections from 2015 and 2016 (unpublished data). *Chrysomya megacephala* is typically only found in very hot, humid environments (Badenhorst and Villet 2018). Despite this, it has been able to successfully make its way as far north as Indiana. Thus, the possibility of *L. cuprina* expanding its distribution in a similar way is not implausible. The overwintering habits of *L. cuprina* in warmer climates consist of quiescence instead of a true diapause stage, and it was shown that transplanted warm-climate strains cannot survive winters in colder climates (Ash and Greenberg 1975a). *Lucilia cuprina* is not believed to be able to overwinter in climates that experience extreme cold, and so it is hypothesized they must 're-migrate' northward in the hottest summer months. This hypothesis aligns well previous studies in which this species was collected in the hottest summer months (Mail and Schoof 1954, Schoof and Savage 1955), as well as with the data collected from Indiana so far, and thus represents the likeliest scenario for *L. cuprina*'s presence in the state. Human-mediated transport via highways has been speculated as an introduction/re-introduction pathway for other insects including the Asian tiger mosquito *Aedes albopictus* Skuse (Diptera: Culicidae) (Medley et al. 2015), and thus may play a role in the range expansion of *L. cuprina* during the summer months. Most collection sites in this study are within a few kilometers of major interstates that form the national hub from which Indiana's state motto was derived: 'The Crossroads of America'. It is possible that *L. cuprina* has resided in at least the southern half of Indiana for a longer period of time than what the present data suggest, as slight changes in climate each year may have made these regions more suitable for these flies in the spring and summer months. However, without high resolution sampling throughout the state and the Midwest as a whole, answers to the questions will remain elusive.

The expanding range of *L. cuprina* in the United States is a concern in the area of forensic entomology. Forensic entomologists routinely use published developmental data sets of insect species found on corpses (i.e., blow fly maggots) to estimate a minimum interval of time elapsed from death of the decedent to discovery of the remains (minimum postmortem interval, PMI_{MIN}) (Amendt et al. 2007). It is vital that the entomologist chooses a species-specific developmental data set that implements similar environmental conditions as those experienced by the wild maggots on the corpse in question. To date, only one developmental study is known to have been performed with a North American strain of *L. cuprina* (Ash and Greenberg 1975b), even though this species is encountered routinely in death investigations in the southern United States, particularly in southeast Texas (Sanford 2017). In fact, only a handful of literature exists worldwide that investigates the developmental physiological requirements for this species (Day and Wallman 2006, Kotzé et al. 2015, Bansode et al. 2016, Bambaradeniya et al. 2017). The three *L. cuprina* data sets compared here give relatively similar development and ADH values (<10.5% error) despite originating from three distant geographical regions. The potential for misidentification of *L. cuprina* maggots in a forensic investigation as *L. sericata* could lead to an under- or overestimation of the PMI_{MIN} by up to 35% if the incorrect species data set is used (Table 3). The ramifications of this error could be significant if used in a court of law. The forensic entomologist should be absolutely certain of the species identification before offering an opinion on the time frame after death. This could be accomplished by getting the second opinion of an expert on the morphological identification of the insects in question, rearing out larval specimens to adulthood (if possible), as well as implementing molecular methods.

Conclusion

Lucilia cuprina is an economically, agriculturally, and forensically important species that should not be ignored in the United States. Given its history as a global pest, as well as its understudied nature in North America, close monitoring of its range expansion should be undertaken. Additionally, the misidentification of this species could be important in a medicolegal context, resulting in a PMI_{MIN} that may be erroneous and fundamentally flawed. More in-depth investigation into North American *L. cuprina* strains is recommended, as population-level data sets would be beneficial to several disciplines. At the very least, closer attention must be paid to detect this sometimes cryptic species, as its distribution may be spreading faster than what is currently known.

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